PHYSIOLOGY OF INSECT DIAPAUSE. XV. THE TRANSMISSION OF PHOTOPERIOD SIGNALS TO THE BRAIN OF THE OAK SILKWORM, ANTHERAEA PERNYI [1]

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The pupa of the oak silkworm, *Antheraea pernyi,* is enveloped in a stout-walled cocoon which gives every impression of being opaque. It seems incredible that an insect in this situation could be influenced by the length of the day and night. Yet such is indeed the case. As demonstrated in the previous paper of this series (Williams and Adkisson, 1964), diapause is persistent at 25° C. when cocoons are exposed to short-day conditions (daily photophases of 4 to 12 hours). By contrast, photophases of 15 to 18 hours provoke the termination of dormancy and the initiation of adult development. It was possible to show that the sensitivity to photoperiod depends on the direct action of light on the pupal brain. In some unexplained manner, light of appropriate wave-length is able to penetrate the opaque cocoon and the pupal cuticle to act on the brain itself.

The phenomenon is examined in detail in the present investigation. All experiments were performed on *A. pernyi.* The cocoons were the diapausing first-brood harvested in late July; on August 27 they were shipped from Japan in a series of cardboard boxes which arrived at Harvard University on September 30. The cocoons were spread on tables and stored at 25 ± 0.5° C. in a room programmed for a daily illumination of 8 hours.

Experimental Results

1. *Photoperiod responses of naked pupae versus pupae in cocoons*

Ninety-six diapausing pupae were removed from cocoons. Forty-eight were placed in an incubator programmed for a daily 8-hour photophase at 25° C.; the other 48 were placed in a similar incubator programmed for a 16-hour photophase. Each incubator also received 92 unopened cocoons from the same lot of material. The cocoons were spread on the shelves and thereby exposed to fluorescent illumination having an average intensity of 175 foot-candles (1883 lux).

The naked pupae were inspected once a week to detect the initiation of adult development in terms of the retraction of the wing epithelium. In the case of the intact cocoons, the emergence of the moths was noted daily; the initiation of

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adult development was computed as having occurred 20 days prior to adult emergence (Williams and Adkisson, 1964).

The results are summarized in Figure 1. During the 8-week term of the experiment, diapause was persistent in all naked pupae and intact cocoons exposed to the short-day regimen (horizontal line in Figure 1). By contrast, the entire series of animals exposed to the long-day conditions initiated development. As indicated by the upper pair of curves in Figure 1, pupae in cocoons responded just as promptly to the long-day stimulus as pupae removed from cocoons. This finding shows that, despite the relatively low intensity of the incident illumination, the presence of the cocoon did not curtail the sensitivity to photoperiod.

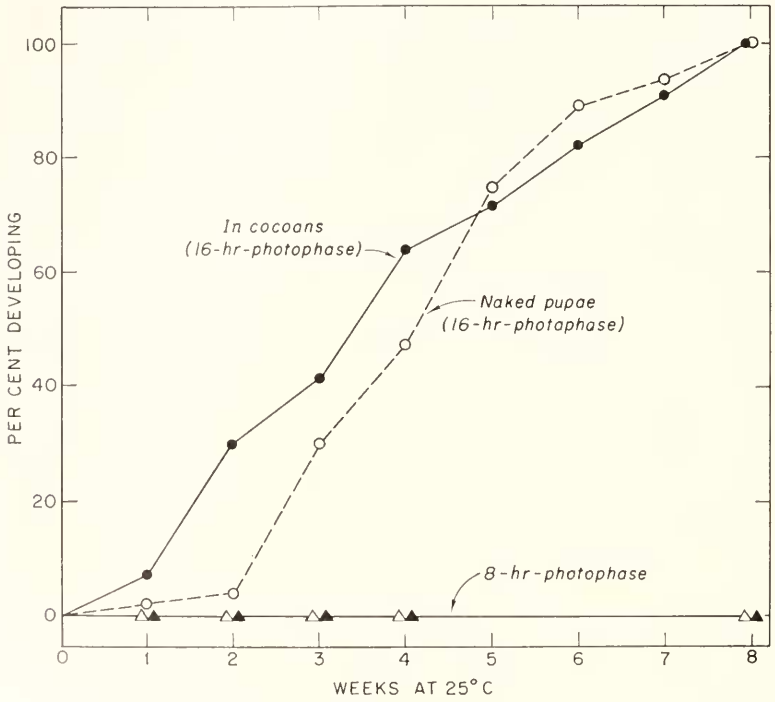


FIGURE 1. The effects of long- and short-day photoperiods on the termination of pupal diapause by naked pupae and by pupae in cocoons. All individuals exposed to the long-day regimen initiated development (*top two curves*). None initiated development when exposed to the short-day regimen (*bottom line*).

2. *Spectral sensitivity of diapausing Pernyi pupae*

Fifty diapausing pupae were removed from cocoons and ten were placed head-up in each of five depressions of an aluminum "muffin tin." Arrangements were made for positioning Corning filters over the depressions. For this purpose each 8×8 cm. filter was sealed with masking tape to a 2-cm. collar cut from a 7.5-cm. thin-wall brass pipe. Each filter was positioned over a specific group of pupae and sealed in place with a gasket of plasticene.

With the filters removed, the entire assembly was placed at 25° C. in an incubator programmed for a daily 16-hour photophase. After 8 hours of exposure to the unfiltered light, the filters were sealed in place so that the pupae received filtered-light during the final 8 hours of the 16-hour photophase. The filters were removed the following morning at the outset of the next photophase. This regimen was repeated daily for 8 weeks.

The five filters, together with their peak transmissions, were as follows: Corning color specification No. 5–62 (398 mμ), No. 5–74 (434 mμ), No. 4–105 (508 mμ), No. 3–110 (580 mμ), and No. 2–78 (640 mμ).

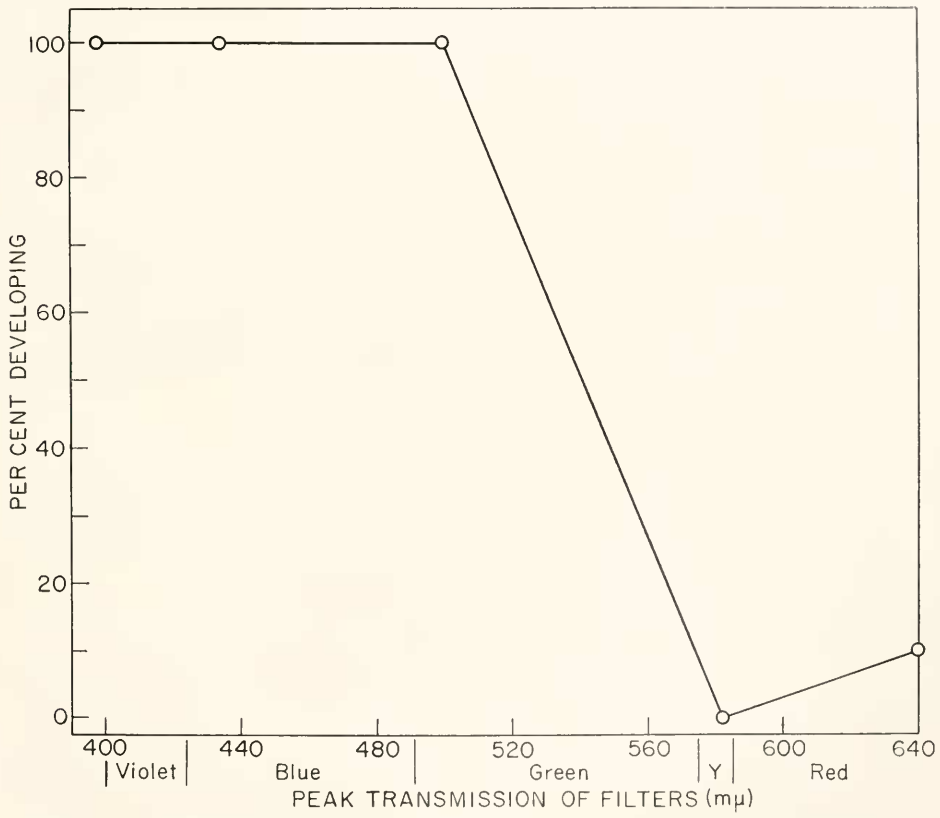


FIGURE 2. The spectral sensitivity of diapausing pupae of *A. pernyi*. The wave-lengths effective in causing the termination of diapause include violet, blue, and blue-green light.

Each of the five groups of pupae was inspected daily in order to detect the initiation of adult development. As controls, two groups of ten pupae were exposed to white light at 25° C. in incubators programmed for 8- and 16-hour photophases, respectively. During the 8-week term of the experiment, these controls behaved as follows: all the animals exposed to white light for 16 hours per day initiated development, while none initiated development under the short-day regimen.

The results observed in the five experimental groups are summarized in Figure 2. Diapause was terminated by all pupae exposed to violet, blue, or blue-green light (398 mμ to 508 mμ); indeed, these animals initiated development as promptly as the control series that received 16 hours of unfiltered light. By contrast, diapause was persistent in all pupae exposed to yellow light (580 mμ) and in all but one of the pupae exposed to red light (640 mμ).

3. β-Carotene-filtered light

Because of the involvement of carotenoids in so many photic reactions, a series of filters was prepared containing graded concentrations of β-carotene dissolved in 95% ethanol. The solution was placed in optical flats prepared by clamping a 0.5-cm. Lucite ring between pairs of glass plates. The filters contained β-carotene in final concentrations of 1000, 100, and 4 parts per million; the densest filter was a deep amber-yellow; the least dense was almost colorless.

The experiment described in the preceding section was repeated using these filters. All individuals initiated adult development at approximately the same rate as controls exposed to a 16-hour photophase of unfiltered light. This shows that β-carotene does not remove all wave-lengths effective in the photoperiod response.

At the conclusion of the experiment, the absorption spectrum of each carotene solution was measured. Even the most concentrated solution showed substantial transmission at wave-lengths higher than 470 mμ. Therefore, the carotene-filtered light contained wave-lengths (470–508 mμ) which were fully effective in implementing the photoperiod response of naked pupae.

4. Spectrophotometric measurements

A series of nine Kodak neutral density filters (0.9 ND) was calibrated for light transmission at wave-lengths ranging from 400 mμ to 700 mμ. A Zeiss M 4 Q III spectrophotometer was utilized for this purpose, the filter being placed in the experimental beam and its transmission measured against air. Transmission by the filters proved to be a function of wave-length and varied systematically from 6% to 11%.

A stack of five ND filters was placed in the experimental beam and its transmission measured against a stack of four filters placed in the reference beam. The difference in light transmission at all wave-lengths corresponded to that of a single filter. This demonstrated that the Zeiss instrument was sufficiently sensitive to measure transmissions by objects as dense as five 0.9 ND filters.

A flattened fragment of a cocoon was positioned in the experimental beam and its transmission compared at each wave-length with that of a stack of four ND filters placed in the reference beam. The transmission of the cocoon was then calculated for each wave-length.

The results summarized in the lower curve in Figure 3 confirm that the cocoon is an extremely dense object to the simple transmission of light; thus, at 400 mμ the transmission was 0.000009%; at 700 mμ, 0.014%. By this same procedure, the transmission was measured for the following fragments of pupal cuticle: (1) the unpigmented facial cuticle; (2) the tan wing cuticle of a palely pigmented pupa; and (3) the black wing cuticle of a heavily pigmented pupa. In these measure-

ments one or two ND filters were placed in the reference beam. The results recorded as the top three curves in Figure 3 show the pupal cuticle to be 5000-fold more transparent than the cocoon to the effective wave-lengths. Moreover, the transmission of blue light (460 mμ) by the facial cuticle was 2 to 5 times that of the wing cuticles.

This fact is illustrated in Figure 4. The anterior third of a Pernyi pupa has here been eviscerated and illuminated from behind to show the transmission of light through the unpigmented facial cuticle and, to a lesser degree, through the tan cuticle of thorax, legs, and antennae.

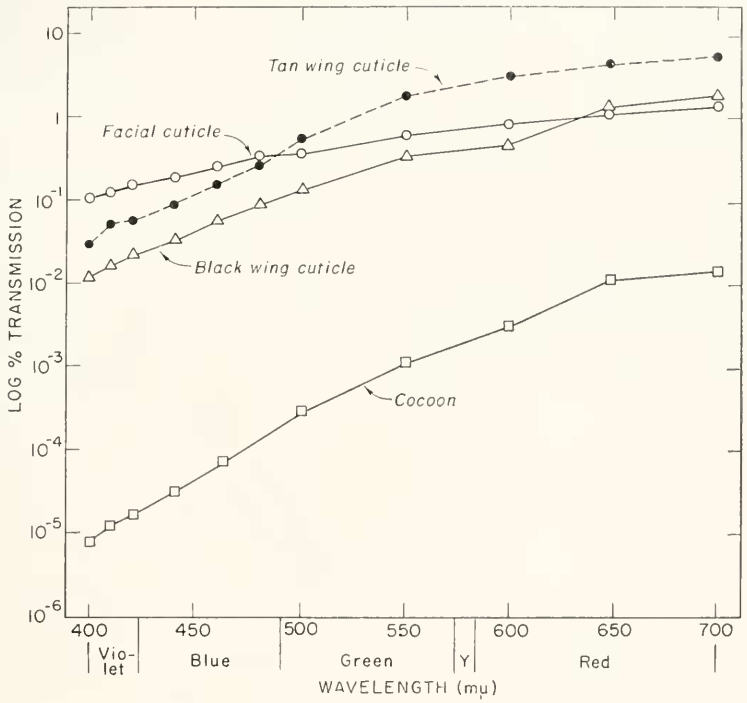


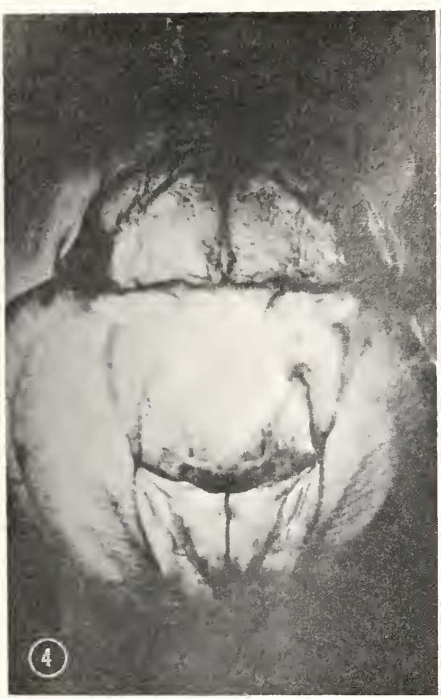
FIGURE 3. Spectrophotometric studies of light transmission by the cocoon (*lower curve*) and by three types of pupal cuticle (*top curves*).

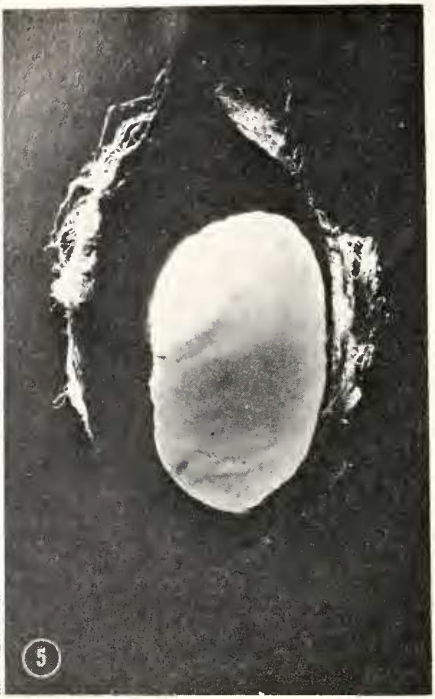
By a combination of the spectrophotometric measurements on the cocoon and the facial cuticle, we calculate that, of blue light (460 mμ) incident on the outside of the cocoon, only 0.0000003% could reach the brain by simple transmission.

5. *The cocoon as a light-integrating sphere*

Since the cocoon is essentially an opaque object in terms of the transmission of unscattered light, the demonstrated light-sensitivity of the pupa must depend on the collection and integration of scattered light (Jacquez and Kuppenheim, 1955).

The geometry of the cocoon proves to be little short of optimal for this purpose. This fact is illustrated in Figure 5 where an empty cocoon has been illuminated





FIGURES 4–7.

from behind by the focussed beam of a 100-watt zirconium arc lamp. By excising the proximal face of the cocoon, one can witness the generalized illumination of the cavity of the cocoon.

This same phenomenon is also illustrated in Figure 6. Here, the light-integrating hemisphere of an incident-light exposure meter has been removed and replaced by the upper half of a Pernyi cocoon. A significant deflection of the meter is observed when the cocoon is illuminated. In order to describe this phenomenon in quantitative terms, the following experiment was performed:

A cocoon was opened by a circumferential razor-blade cut around its long axis. After the removal of the pupa and the old larval skin, a micro-photocell (Texas Instruments LS-222) was suspended in the cavity of the cocoon. The latter was reassembled and sealed with Duco cement and a narrow band of opaque tape; the paired leads to the photocell passed to the outside through the incision.

The cocoon was suspended in a dark room at a standard distance from a microscope lamp (Bausch and Lomb No. SVB-73). The light first traversed a water-filled filter, 3 cm. in depth, and then illuminated one entire 180° hemisphere of the cocoon. The output of the photocell was measured in millivolts. The measurements were repeated with the cocoon oriented at various attitudes with reference to the incident white light. The output of the photocell varied from 120 to 270 millivolts, depending on the orientation of the cocoon, the average being about 240.

The photocell was then removed from the cocoon and illuminated directly by the same white light at the same standard distance. The output was now 400 millivolts. Neutral density filters were then placed in the light beam to lower its intensity. A sandwich of two filters, each having 10% transmission for white light, decreased the photocell output to 240 millivolts. So, in rough terms, we can say that about 1% of the incident white light reached the photocell when the latter was inside the cocoon.

6. *Light-integration as a function of wave-length*

The preceding experiment was repeated with interference filters placed in the beam to establish monochromatic light. A total of nine wave-lengths was examined in this manner. Then with the photocell removed from the cocoon, the latter's properties at each wave-length were equated to that of the calibrated ND filters.

The results are recorded as the upper curve in Figure 8. The light-integrating properties of the cocoon prove to be substantial, especially in the range 440 mμ to 510 mμ.

FIGURE 4. The anterior third of a Pernyi pupa has been eviscerated and illuminated from behind by white light. The photograph illustrates the transmission of light by the unpigmented facial cuticle and, to a lesser degree, by the tan cuticle of the thorax, legs, and antennae.

FIGURE 5. An empty cocoon is here illuminated from behind by an intense, focussed spot of white light from a 100-watt zirconium arc lamp. By excising the proximal face of the cocoon, one can witness the collection of scattered light within the cavity of the cocoon.

FIGURE 6. A hemisphere of Pernyi cocoon serves as a light-integrating sphere when substituted for the integrating hemisphere of an incident light exposure meter.

FIGURE 7. A micro-photocell has been sealed into a Pernyi pupa with its light-sensitive element placed just beneath the unpigmented facial cuticle. The pupa was then sealed inside the cocoon to permit measurements of the light energy reaching the brain at each wave-length.

7. Light reaching the brain

The cuticle overlying the legs was excised from an anesthetized pupa. The micro-photocell was then implanted through this mid-ventral area so that its light-sensitive element was positioned precisely beneath the transparent facial cuticle, *i.e.*, in the place normally occupied by the brain. The photocell was held

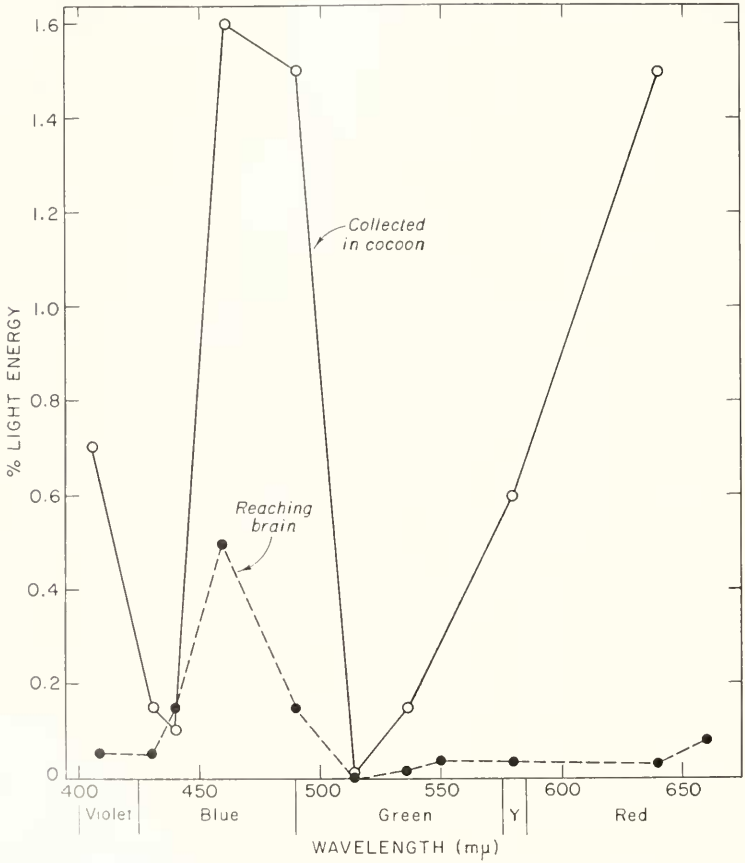


FIGURE 8. The effect of wave-length on the per cent of incident light energy which penetrated the cocoon (*solid-line*) and which reached the brain of a pupa sealed within a cocoon (*hatched-line*). The entire system shows a conspicuous "window" centering around 460 mμ.

in place and the wound sealed by the application of opaque cement (see Fig. 7). The pupa was then sealed within a cocoon as described above.

The measurements described under Section 6 were repeated with the results summarized by the lower curve in Figure 8. The optical properties of the entire system prove to be maximally effective for blue light. Thus, in the range 440 to 490, more than 0.14% of the light energy reached the brain; indeed, at 460 mμ, no less than 0.5% of the scattered light was recollected to act on the brain.

Discussion

1. *Spectral sensitivity of the pupal brain*

In the previous paper of the series (Williams and Adkisson, 1964), light was found to act directly on the pupal brain to control the secretion of brain hormone. According to the present study, the effective wave-lengths extend throughout the lower third of the visible spectrum to include the violet, blue, and blue-green (398 mμ to somewhat above 508 mμ). Yellow or red light (580 mμ to 640 mμ) was without significant effects. The lowest wave-length tested (398 mμ) was fully effective; consequently, we are unable to state how far the spectral sensitivity extends into the ultraviolet.

In order to have any effect, the pertinent wave-lengths must be absorbed. And since the effective wave-lengths include the lower third of the visible spectrum, the absorption of light is evidently accomplished by a pink brain pigment.

The spectral sensitivity of the Pernyi brain is in close agreement with that described for the mite, *Metatetranychus ulmi* (Lees, 1955), and for certain, but by no means all, insects that have been studied (for reviews, see Lees, 1955; Bünning, 1960, 1964; Farner, 1961; de Wilde, 1962; Danilevskii, 1965).

The Pernyi pigment therefore differs from the phytochrome system of the higher plants in terms of the latter's sensitivity to red and far-red (Borthwick, 1959; Hendricks, 1959). However, the photoperiodic responses of fungi (Bünning, 1964) and the phototropic reactions of higher plants (Shropshire and Withrow, 1958; Withrow, 1959) show a spectral sensitivity which is essentially the same as that of the Pernyi brain.

The phototropic reactions of plants are thought to depend on the absorption of blue light by a carotenoid or flavin pigment. In the present investigation, β-carotene was tested and found to be inappropriate in the insect system because of its scant absorption of certain wave-lengths (470–508 mμ) which were fully effective in the photoperiod response. However, it may be noted that numerous other carotenoids, such as the arthropod pigment, astaxanthin, show absorption spectra which include these higher wave-lengths (Karrer and Jucker, 1950).

2. *Optical properties of the cocoon and the pupal cuticle*

In order to be absorbed by the pink brain pigment, light must first traverse a series of barriers before reaching the brain; namely, the wall of the cocoon, the pupal cuticle, the underlying epidermis, and a narrow zone of hemolymph. Due to its opacity the cocoon is the major obstacle to the simple transmission of light (Fig. 3). The pupal cuticle is, by contrast, 5000 times as transparent as the cocoon. Moreover, the measurements performed on the several categories of pupal cuticle show that, at 460 mμ, the unpigmented facial cuticle is twice as transparent as tan wing cuticle and five times as transparent as black wing cuticle (Fig. 3).

3. *Light-integration by the cocoon*

Despite its opacity, the cocoon proves to be an effective vehicle for the collection and integration of scattered light ("haze"). The blue haze collected within the cavity of the cocoon then penetrates the pupal cuticle to act on the brain. Analogous

biological systems have been discussed in detail by Shibata (1958) and French (1959).

Our measurements reveal the surprising fact that the entire system is remarkably effective in the collection, integration, and transmission of blue light ranging from 440 mμ to 490 mμ. As noted in Figure 8, a particularly prominent "window" centers around 460 mμ. All these wave-lengths, as mentioned above, are fully effective in the photoperiod reaction.

4. *Light intensity*

In the experiments reported here, no attempt was made to obtain a true action spectrum in terms of the energy threshold of the brain as a function of wave-length. The fluorescent lamps in our incubators saturated the brain's photoreceptive mechanism even though the incident illumination on the outside of the cocoons was only 175 foot-candles (1883 lux)—*i.e.*, about 2–5% the intensity of direct sunlight.

Spectroscopic examination of the fluorescent lamps revealed three intense emission lines (435.8 mμ, 546.1 mμ, and 577.0 mμ) of which only the one at 435.8 mμ falls within the spectrum shown to be effective in the photoperiod reaction. If we assume that, of the total light acting on the outside of the cocoon (175 foot-candles), about 25% is contributed by effective wave-lengths, the intensity factor is reduced to 44 foot-candles. Of this incident intensity, not more than about 0.2% could reach the brain after traversing the several barriers (Fig. 8). Hence we may say that the brain's photoreceptive mechanism is fully saturated by intensities not in excess of about 1 foot-candle (10.8 lux) of blue light. This value is in line with those reported for other insects where saturating intensities ranging from 0.01 to 10 foot-candles (0.11–108 lux) have generally been encountered (see Lees, 1955; Farner, 1961; de Wilde, 1962).

Summary

1. The pupal diapause of *Antheraea pernyi* is sustained by exposure of cocoons to short-day conditions (daily photophases of 4 to 12 hours) and terminated after exposure to daily photophases of 15 to 18 hours.

2. The photoperiod signal is conveyed by the direct action of violet, blue, and blue green light (398–508 mμ) on the brain itself. This finding implicates a pink brain pigment in the absorption of the effective wave-lengths.

3. Despite its opacity, the cocoon functions as a light-integrating sphere in the collection of scattered light—especially of blue light ranging from 440 mμ to 510 mμ.

4. After its collection within the cavity of the cocoon, the blue "haze" penetrates the pupal cuticle to act on the brain. The brain's photoperiod mechanism is "saturated" by less than 1 foot-candle of blue light.

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